mTOR in Aging and Age-Related Diseases

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Abstract

Caloric Restriction (CR), a dietary regimen that delays aging and the onset of age-associated diseases, induces a reprogramming of energy metabolism that may be important in its mechanisms of action. In mice, the effect of CR on adipose tissue is particularly striking and presents a possible means to integrate the CR response among tissues through changes in adipose tissue-derived systemic signaling. Liver is also ameliorated by treatment with rapamycin, a potent inhibition of nutrient-sensitive growth regulator mammalian target of rapamycin (mTOR). It is unclear if mTOR plays a role in CR or if rapamycin, like CR, impacts mitochondrial function in adipose tissue.

Here, we show that rapamycin treatment regulates proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), a master regulator of mitochondrial energy metabolism. Rapamycin treatment increased inhibitory phosphorylation of glycogen synthase kinase 3 beta (GSK3β), a key factor in PGC-1α protein degradation and regulation within hours of treatment in adipose tissues (fat cell precursors). Treatment with rapamycin was also associated with alterations in PGC-1α activity as indexed by increased mitochondrial membrane potential and enhanced respiration, both indicators of activated energy metabolism. We next looked at mature adipocytes. Rapamycin inhibits the processes of differentiation and proliferation of adipocytes. Our data demonstrate that PGC-1α and PGC-1δ are differentially regulated during differentiation and that key transcription factors in activity were disrupted in rapamycin-treated cells. Importantly, activation of PGC-1α and inhibition of GSK3β directly and negatively impact fat storage in mature adipocytes. Consistent with this, adipocytes overexpressing recombinant PGC-1α also exhibit impaired fat storage. The physiological relevance of these findings was confirmed in vivo, where stabilization of PGC-1α and inhibition of GSK3β was also observed in adipose tissue from rapamycin-treated mice. These data confirm that rapamycin, like CR, activates PGC-1α in adipose tissue. These data show that activation of PGC-1α-regulated mitochondrial energy metabolism in adipose tissue is a conserved feature of delayed aging. Our findings indicate that treatments to activate mitochondrial energy metabolism may be valuable in the context of age-associated diseases such as diabetes, neurodegenerative diseases, and cancer.

Background

Caloric Restriction Rapamycin

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Altered Metabolism

Longevity

Study Design

Initiate diet Control Rapamycin

Harvest Tissues Analysis Control Rapamycin

Initiate treatment Harvest cells Analysis Unrelated Rapamycin

Rapamycin alters mitochondrial metabolism in preadipocytes

Figure: Short term treatment with rapamycin in 3T3-L1 preadipocytes leads to enhanced inhibitory phosphorylation of GSK3β, a key regulator in PGC-1α turnover, and increased mitochondrial activity.

A. Immunoblot detection of protein levels in 3T3-L1 preadipocytes treated with rapamycin (10 nM) for the indicated time.
B. JC-1 assay in 3T3-L1 preadipocytes in the presence of rapamycin (10 nM). *p<0.05
C. Schematic of PGC-1α regulation by GSK3β

Rapamycin disrupts regulation of PGC-1α during adipogenesis

A. Unrelated Rapamycin

B. Rapamycin

Figure: Temporal regulation of PGC-1α and GSK3β during adipogenesis is disrupted in the presence of rapamycin. Immunoblot detection of protein levels in 3T3-L1 cells during the process of differentiation shows temporal alterations in metabolic regulators.

Alterations in PGC-1α impede lipid storage

A. Untreated Rapamycin

B. Rapamycin

Figure: Lipid accumulation during adipogenesis in 3T3-L1 adipocytes. Cells were fixed at day 9 of differentiation and stained with ORO to determine lipid content. Cells stimulated to differentiate in the presence of rapamycin or while overexpressing the PGC-1α gene failed to accumulate lipid to the levels of untreated and vector control cells.

Conclusions

- Rapamycin increases inhibitory phosphorylation of GSK3β and enhances activity of PGC-1α-dependent processes
- Rapamycin disrupts GSK3β-mediated regulation of PGC-1α within adipocytes during differentiation
- Inappropriate levels of PGC-1α impacts lipid accumulation
- Protein levels of PGC-1α and its regulator, GSK3β, are altered in adipose tissue from rapamycin-fed mice

Here we show that rapamycin impacts mitochondrial metabolism in adipose tissue. We propose that the interaction between mTOR signaling and PGC-1α through GSK3β is a conserved feature of aging and age-associated disease risk.

References

Schieke S, Phillips D, et al. The mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption and oxidative capacity.

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